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IV. EXPERIMENTAL RESULTS IN ISCHNURA AND ENALLAGMA.

BY JOSEPH HALL BODINE.

(The following paper was written out by Mr. Bodine in nearly the form here presented. Owing to his having entered the war service of the United States, it is unlikely that he will be able to continue these researches for many months. He has placed his manuscript in my hands with the wish that I do with it as I think best. I have made some slight verbal changes, but otherwise the paper represents the author's own observations and conclusions.—*Philip P. Calvert.*)

The problem of the physiology of respiration among insects is by no means a settled one and presents a great many obstacles to its solution, especially in certain aquatic stages.

The types, theories, etc., of respiration found in insects have been much discussed and can be easily learned from any of the more recent text-books of entomology and comparative physiology and hence need not be taken up here. I shall deal entirely with the phenomena among the dragonflies, restricting the problem to the suborder Zygoptera.

The morphological studies upon the structure of the rectum and caudal gills of the larvæ of Zygoptera bring forward several questions, as to the exact function of these parts.

The organs generally supposed to be concerned in the respiration of these larvæ are the caudal tracheal gills, the so-called rectal gills or folds, the body surface and the spiracles. The structure of the rectal gills or folds, especially as regards the distribution of tracheæ to the same is discussed in the papers of Calvert, Jamieson, Cullen and Carroll, and need only be referred to here. (The structure of the caudal gills has been described by Tillyard in 1917, in a paper published after Mr. Bodine's work ceased.—P. P. C.)

Various theories of respiration for these larvæ have been proposed, some of which are quite conflicting and the remainder are based rather upon philosophical, than physiological, ideas.

Briefly stated and outlined these theories are as follows: Réaumur (1742) and Roesel von Rosenhof (1749) regarded the caudal gills

as having a fin-like or rudder-like function, mentioning no respiratory function whatever. Dufour (1852) and Roster (1886) ascribed to them the functions of respiration and locomotion. Sharp (1895), remarking that the nymphs lived after removal of the caudal gills, thought their respiratory function must be of an accessory nature. Heymons (1904) held that the entire body surface serves for respiration in addition to the gill-appendages.

A respiratory function was first attributed to the rectum in this group by Roesel von Rosenhof (1749). Dufour (1852) described the gills in the rectum and from this time on a respiratory function has often been assigned to them. Calvert (1915) has given a detailed account of the manner in which water is taken into and expelled from the rectum, and from these observations, we must undoubtedly ascribe to the rectum a relation to the respiratory functions of the nymph. Tillyard (1916) asserts that in early larval life, immediately after hatching, for a period of half an hour or so, the rectum contracts vigorously and to these contractions assigns a respiratory function, especially since he thinks the caudal gills of the very young larvæ are incapable of carrying on respiration. This conclusion is undoubtedly true, but in later periods of the larval life, he attributes to these intermittent contractions only a possible respiratory function, governed "by the efficacy of the total of other respiratory activities. Respiration through caudal gills, through lateral abdominal gills when they occur and through the integument in such cases as it may occur, all differ from rectal respiration in being practically continuous, carried on without special efforts on the part of the larva. Consequently, if the caudal gills have a poor tracheal supply, or a very tough integument, or if the general integument of the larva be unsuitable for respiration by diffusion, the call on the larva for rectal pulsations must be heavier."

If these statements be true, larvæ which are old and hence have thicker chitin over body and gills ought to show rectal pulsations to a greater degree than the normal larvæ. Such a condition, however, has not been found to be the case in the larvæ examined, but such larvæ have contractions quite like those younger and with softer chitin. Tillyard continues: "If the caudal gills are accidentally lost, we should expect that the rate of rectal respiration would be increased." When the gills are removed one at a time, or all together, no change in rate of rectal respiration has ever been noticed, hence we can be quite sure of the non-respiratory function of these caudal gills.

Tillyard's earlier views (1915) as to the respiration of the Zygopterous larvæ are stated as follows: "Firstly, I shall deal only with the suborder Anisoptera, *i. e.* those dragonflies whose larvæ breathe by means of rectal gills, and shall not consider the somewhat different problem presented by the *Zygoptera, whose larvæ breathe by means of caudal gills.*" (The italics are ours.)

Calvert (1915) says, "From the various data which have been brought together here, it seems reasonable to suppose, at least until much more exact experiments show the limitations of each mode of respiration for different stages and for different species of Zygopterous larvæ, that the general body surface, the caudal processes, the rectal epithelium, certain spiracles and in a few species lateral external abdominal tracheal gills, all contribute to satisfying the needs of the organism for oxygen."

Summing up this historical treatment of the possible functions of these parts in respiration we find that three methods for the taking in of oxygen by the larva are described, (1) by the caudal tracheal gills—(2) by the rectal gills—(3) by the surface of the body (including spiracles).

Each of these will be discussed in turn and reasons for considering them heretofore as entering into the phenomena of respiration, will be given.

I. *The caudal tracheal "gills."* These, three in number, situated on the caudal end of the abdomen, have a structure which seems admirably adapted for a respiratory function. Normally, they are present on the larva throughout its life, but are, at the time of transformation, left behind on the exuvia in their normal position and relation. Their presence, however, does not seem to be necessary, because larvæ with one, two or all of them missing, live apparently normally and transform into the adult in the usual manner. Hence, if they do act as respiratory organs, it must be only in a secondary manner. They do, however, aid the larva in swimming, acting as fins or as rudders. When at rest the larvæ have the tendency to move the abdomen in a wave-like fashion from side to side, the "gills" in this case possibly acting only as mechanical devices for the stirring up of the water and hence in bringing water in which the content of oxygen is richer near the larva.

If they have (as many hold) a respiratory function, how can the larva continue to live and reach maturity, when they are completely removed? This seems to entirely eliminate them (or at least to cast much doubt on their rôle) in a consideration of

respiration. Other organs must take up a respiratory function after the removal of the caudal "gills" from the body, but this idea is scarcely conceivable since it would be almost impossible for the animal to adjust any such structures in so short a time. Since these other organs seem especially adapted to a respiratory function, their existence makes the possible respiratory function of the caudal gills seem quite improbable.

II. *The rectal folds or gills.* The structure of these, associated with the observed intake and outgo of water into the rectum through the anus, seems to warrant their inclusion in the consideration of possible respiratory functions. The only other function which could be assigned to the intake and outgo of water into and from the rectum is that of a locomotor one. This, however, is rather improbable for several reasons. These larvæ, when moving in the water, do so by a worm-like wriggling of the body and do not "shoot" through the water as do the Anisopterous larvæ where the rectum does have a locomotor function. The water when expelled from and taken into the rectum does not create currents strong enough to propel the larva through the water. They are produced when the larva is at rest and in this case do not move it.

The function of respiration, then, can be assigned to the rectum, until another possible means for this function can be found. The possible respiratory function of the skin will have to be considered before one can draw a final conclusion, however.

III. *The skin.* The skin, or hypodermis and chitin, of the larvæ, varies in thickness and hardness according to the length of time from the preceding moulting period. Just after exuviation the chitin is soft and quite transparent. Soon it becomes hard, thickens and becomes quite dark in color. Ramifying throughout the surface of the larva are innumerable, fine, thread-like tracheæ which join larger branches and finally these latter unite with the main tracheal trunks. The thread-like tracheæ being found over the entire surface of the larva's body, afford a very large area exposed directly to the water by which the larva is surrounded. Thus the structure of the skin seems to warrant its consideration as a means of respiration.

IV. *Spiracles.* Spiracles on the thorax and abdomen are supposed to function when the larvæ crawl up out of the water into the air. This idea, perhaps true, need not be considered here because we shall only deal with respiration as carried on when the larvæ are actually submerged. However, when the larvæ come to the

surface, and especially at the time of transformation, respiration must be carried on by this method, since the larvæ have then come to the end of their aquatic existence and no longer need those means of respiration previously employed but rather are ready to begin an aerial existence, hence respiration by means of spiracles.

Experiments.

Various experiments made during 1916 and 1917, seem to throw some light upon the possible respiratory mechanisms of Zygopterous larvæ. The larvæ used were of species of *Ischnura* and of *Enallagma*, obtained from small ponds at Primos, Pennsylvania. Many individuals were employed, the experiments were repeated several times and the data for each set of experiments represent average results.

The normal larva (with three caudal "gills") when observed under a binocular microscope and in a Syracuse watch glass, show rhythmic pulsations in the rectum, in the manner described by Calvert (1915) for the larva of *Hetaerina americana*, *i. e.*, each pulsation consisted of three to four successive contractions of the rectum, followed by a pause. No uniformity, however, in the number of contractions per minute seemed to exist; this was possibly due to age, size and environment of the larvæ under examination. Particles of carmine and of lampblack were also used and these were seen to go in and out of the rectum.

The current of water taken in and expelled was not so forcible that a possible locomotor function could be ascribed to it. The currents were not strong but rather weak and of only sufficient strength to be taken into and expelled from the rectum so that a change of water could be brought about.

The three caudal gills were then removed as follows: the larva was placed under a binocular microscope as above and a fine needle, bent at a right angle was used to remove the gills. The needle was placed on the particular gill to be taken off and the larva pulling away under this stimulus, broke off the gill at its attachment to the posterior end of the abdomen. By this method the three gills were successively removed. The results obtained were the same whether one, two or three gills were removed at the same time or at intervals. The rate of rectal contractions was not increased or decreased at the time of operation and several hours afterward the contractions had not changed their regular normal rhythm.

These larvæ, after the removal of the gills, behaved normally and lived in a regular way, and moulted as did larvæ with gills.

To determine whether the larvæ breathed through the skin several experiments were conducted. Glass battery jars were filled with pond water and into them were put both larvæ with gills and also those from which gills had been removed. A fine gauze was then stretched across the jars about two inches below the water's surface, so that the larvæ could not come up to the air. Larvæ kept under these conditions, as well as those kept in jars from which the gauze was absent, lived for periods of four to six days—depending of course, upon the amounts of available food substances present in the water. The food question, however, can be eliminated, because both the larvæ in the controls, as well as those under experiment, were kept in water obtained from the same pond and were under similar laboratory conditions.

The same experiment, performed in a slightly different way, gave like results. Larvæ, both with and without gills, were put in vials, the ends of which were covered with gauze, and the vials were then dropped into battery jars containing pond water. Larvæ put into distilled water in the same manner as above, lived only one to two days—in all probability due to food and osmotic conditions of their environment.

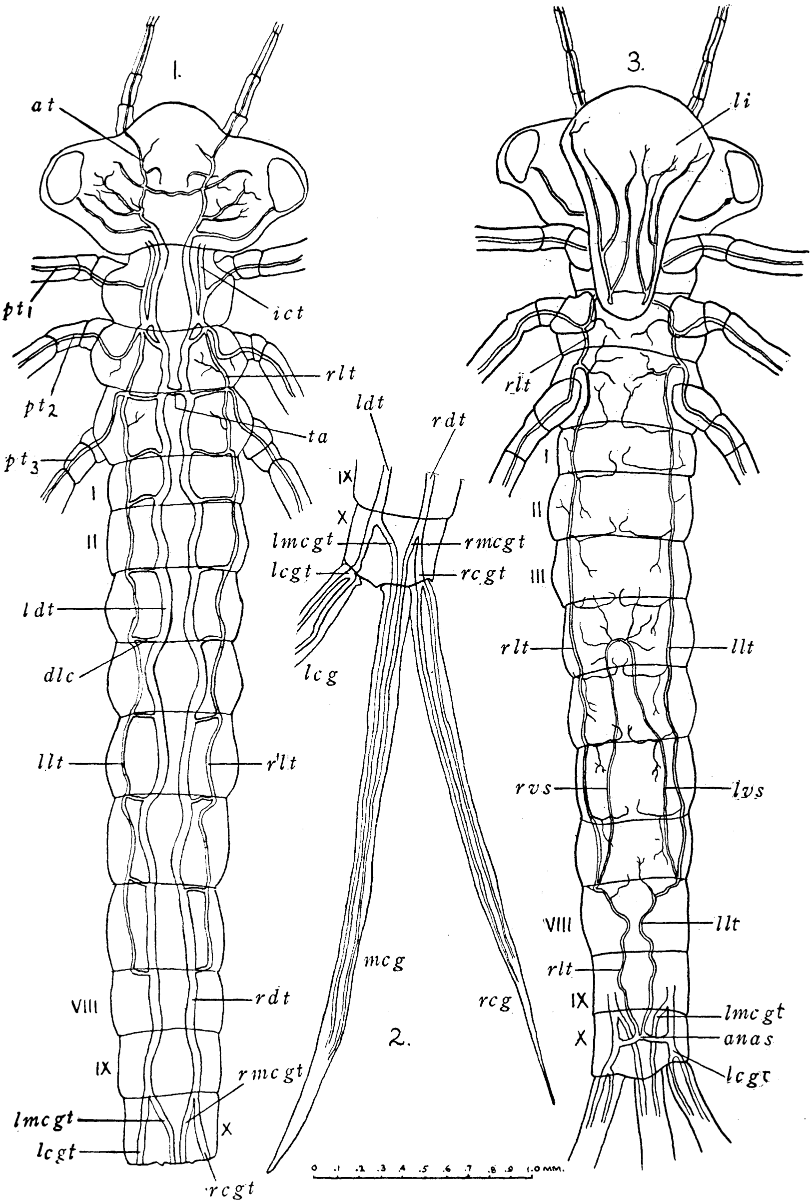
When the water was previously boiled the length of life of the larvæ was greatly reduced—to one and one-half days when under the above conditions. This is doubtless due to lack of food and oxygen in the water used.

Tracheal System of larva of *Enallagma* sp., from Primos, Delaware County, Pennsylvania. Larva No. 101. Drawings made by J. H. Bodine, November 12, 1915. Fig. 1, dorsal view. Fig. 2, dorsal view of hind end of abdomen with caudal gills. Fig. 3, ventral view. From a transparent specimen. (These are, perhaps, the first complete views, dorsal and ventral, of the tracheal system of an Agrionine larva to be published. A highly magnified drawing of the distal part of a caudal gill in lateral view was published by C. G. Carus as long ago as 1827.—P. P. C.)

Abbreviations.

anas, anastomosis of tracheæ in abdominal segment X.
at, antennal trachea.
dlc, transverse trachea connecting dorsal and lateral longitudinal tracheæ.
ict, inferior cephalic trachea (to maxilla and labium).
lcg, left caudal gill.
lcgt, left caudal gill trachea.
ldt, left dorsal trachea.
li, labium.
llt, left lateral trachea.
lmcgt, left median caudal gill trachea.
lvs, left visceral trachea.
mcg, median caudal gill.
pt1, *pt2*, *pt3*, tracheæ of 1st, 2nd and 3rd legs, respectively.
rcg, right caudal gill.
rcgt, right caudal gill trachea.
rdt, right dorsal trachea.
rlt, right lateral trachea.
rmcgt, right median caudal gill trachea.
rvs, right visceral trachea.
ta, thoracic anastomosis.

The Roman numerals denote the numbers of the abdominal segments.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From these experiments we see, that respiration must be carried on by one of two methods—(1) by the rectum or (2) through the integument. The probability of the passage of the air of the water, through the chitin of the body is quite doubtful. The larvæ used were for the most part of rather hard, thick chitin and the passage of the air through this would be very slow and in all likelihood too small in amount to serve the entire respiratory needs of the larvæ. The possibility of the larvæ getting near or on the surface of the water and taking in air through the spiracles present on the thorax or directly through the skin is excluded, since the larvæ could not get to the surface. The only method left, by which the air could have been taken in, was by means of the rectum. The manner in which the air is taken out of the water by the larval rectum is one of dispute and need not be taken up here. Tillyard (1915) has admirably discussed this question and reference to his work will show the present views.

In experiments made with other objects in view, viz., the action of certain gases or fumes of different chemicals, *e. g.*, ether, chloroform, etc., upon the heart's action, very interesting phenomena were observed with respect to their effects upon the contractions of the rectum. The details of these experiments will not be given now, but their effects on the respiratory phenomena in which we are interested will be considered.

Before the various gases affect the action of the heart, violent contractions of the rectum always precede and very shortly afterward the effects upon the heart's action can be observed. In an animal which has been under the influence of the gas and is recovering, similar contractions of the rectum can be observed before the heart again beats normally. These observations would seem to show that the gas is first taken into the rectum and distributed from it to the other parts of the body. The contractions, when the animal is recovering, would also seem to indicate that air must be carried to the tissues before normal activities could again be resumed.

If the larvæ breathe through the skin, these contractions of the rectum would be quite superfluous. They cannot be due to the action of the gas used, as when the larva was recovering, it was kept in normal atmosphere, away from the effects of the gas. Hence the rectum must in some way act as a respiratory center for the larvæ.

The distribution of the tracheæ, and the general structure of the rectum of the larvæ employed in these experiments correspond

fairly closely to that of the other species studied in the papers by Cullen, Jamieson, Carroll and Calvert.

Whether the air which enters the rectum is carried from it by the tracheæ or whether it diffuses through into the spongy tissue in contact with the basement membrane of the rectum and is then carried by the blood to the different parts of the body, is at present, rather difficult to say on purely physiological grounds and will require more intensive study.

From a morphological study of the species of larvæ used in my experiments, carried out in a way similar to those of Calvert, Jamieson, Cullen and Carroll, I find that the number of tracheæ and of tracheoles, distributed to the rectum is very small—much too small to supply or take up sufficient oxygen for the respiratory needs of the larvæ. Doubtless the greater part of the oxygen diffuses through the spongy bands as suggested by Carroll.

If oxygen from air in the water diffuses through the walls of the rectum and is taken up by the blood and is thus distributed to the various parts of the body, the tracheæ and tracheal trunks must be accessory structures, since otherwise the oxygen would have to be taken up by them from the blood and be distributed by them to the various parts of the body. This is hardly conceivable, since the blood is found to circulate freely to all parts of the body. One fact which would point to such a function of the blood is, that the heart's action when subjected to different gases, is not interfered with until after violent contractions of the rectal wall have taken place, showing that possibly the gas is carried directly to the heart by means of the blood and not by means of the tracheæ. On the other hand, from morphological studies already cited (Calvert, Cullen, Jamieson and Carroll) we see that the tracheal supply to the rectum and especially the manner of ending of the tracheoles, would seem to indicate the possible diffusion of the oxygen through other places than those where these tracheoles end (Carroll). If such a condition is found, the problem becomes one quite different than heretofore supposed—the tracheoles and tracheæ then must play only a secondary rôle in the distribution of oxygen to the body if at all; the blood must be the important means of transportation of oxygen either to the tracheæ, or directly to the various parts of the body.

Tillyard (1916) says regarding the mode of diffusion of air in Zygopterous larvæ: "The diffusion of CO^2 outwards into the rectal water supply, and the diffusion of air inwards, is undoubtedly effected

through the rectal epithelium *without any specialisation of the tracheal supply of that region*, and the exchange must be regarded as being primarily one between the blood of the larva and the water in the rectum, quite irrespective of the tracheal system, which is bound to benefit sooner or later by the change." His idea based almost entirely upon speculative grounds, perhaps can be better justified after the detailed morphological work on the tracheal supply has been done. His attempt, however, to correlate the thickened folds of rectal epithelium of Zygopterous larvæ with the basal pads of Anisopterous larvæ, especially as regards the elimination of carbon dioxide, are, perhaps, not so well founded. The detailed morphological work has shown that these folds are the definite cellular parts of the rectum and have a tracheal supply. The great quantities of spongy tissue present between the folds suggests the supposed function attributed by Tillyard to the folds themselves, viz., the elimination of carbon dioxide.

Conclusions.

1. Zygopterous larvæ breathe by means of the rectum from the time of hatching until transformation.
2. Caudal "gills" serve only in a mechanical way, as rudders in the locomotion of the larvæ.
3. Respiration through the skin of the larva is doubtful, but if it takes place, is only in a very slight degree, not supplying enough oxygen for the respiratory needs of the larva.
4. Regular rhythmic contractions of the rectum take place throughout the aquatic life of larvæ.
5. Rhythm of rectal contractions is not interfered with by removal of the caudal "gills."

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